

099656-10401

[0045] Monoclonal antibodies to a particular polypeptide, for example, IL-4, IL-8, IL-13 and others, can be used in immunoassays, such as in liquid phase or bound to a solid phase carrier, to detect polypeptide associated with a disorder, such as dermatitis. In addition, the monoclonal antibodies in these immunoassays can be detectably labeled in various ways. Examples of types of immunoassays which can utilize monoclonal antibodies of the invention are competitive and non-competitive immunoassays in either a direct or indirect format. Examples of such immunoassays are the radioimmunoassay (RIA) and the sandwich (immunometric) assay. Detection of the polypeptide antigens using the monoclonal antibodies of the invention can be done utilizing immunoassays which are run in either the forward, reverse, or simultaneous modes, including immunohistochemical assays on physiological samples. Those of skill in the art will know, or can readily discern, other immunoassay formats without undue experimentation. In addition, there are a number of commercially available antibodies to cytokines of interest.

[0046] The term "immunometric assay" or "sandwich immunoassay" includes simultaneous sandwich, forward sandwich and reverse sandwich immunoassays. These terms are well understood by those skilled in the art. Those of skill will also appreciate that antibodies according to the present invention will be useful in other variations and forms of assays which are presently known or which may be developed in the future. These are intended to be included within the scope of the present invention.

[0047] Monoclonal antibodies can be bound to many different carriers and used to detect the presence of a cytokine polypeptide. Examples of well-known carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses and magnetite. The nature of the carrier can be either soluble or insoluble for purposes of the invention. Those skilled in the art will know of other suitable carriers for binding monoclonal antibodies, or will be able to ascertain such using routine experimentation.

[0048] A cytokine polypeptide may be detected by the monoclonal antibodies when present in biological fluids and tissues. Any sample containing a detectable amount of cytokine can be used. A sample can be a liquid such as blood, serum and the like, or a solid or semi-solid such as

tissues, skin sample, or, alternatively, a solid tissue such as those commonly used in histological diagnosis.

[0049] In performing the assays it may be desirable to include certain "blockers" in the incubation medium (usually added with the labeled soluble antibody). The "blockers" are added to assure that non-specific proteins, proteases, or anti-heterophilic immunoglobulins to anti-cytokine immunoglobulins present in the experimental sample do not cross-link or destroy the antibodies on the solid phase support, or the radiolabeled indicator antibody, to yield false positive or false negative results. The selection of "blockers" therefore may add substantially to the specificity of the assays.

[0050] It has been found that a number of nonrelevant (*i.e.*, nonspecific) antibodies of the same class or subclass (isotype) as those used in the assays (*e.g.*, IgG1, IgG2a, IgM, etc.) can be used as "blockers". The concentration of the "blockers" (normally 1-100 $\mu\text{g}/\mu\text{l}$) may be important, in order to maintain the proper sensitivity yet inhibit any unwanted interference by mutually occurring cross reactive proteins in the specimen.

[0051] In another embodiment the invention provides a kit for non-invasively obtaining samples from the skin comprising a cell collection device selected from the group consisting of a rigid surface, an adhesive tape, or both and a cell lysis buffer suitable for preserving nucleic acids in the skin sample. In another embodiment the invention provides a kit comprising a cell collection device, a cell lysis buffer and an mRNA detection reagent for distinguishing irritant and allergic reactions in a tissue. The kit comprises a polynucleotide detection reagent, for example an oligonucleotide primer that is complementary to a polynucleotide sequence encoding a cytokine, such as IL-4. Such a kit may also include a carrier means being compartmentalized to receive in close confinement one or more containers such as vials, tubes, and the like, each of the containers comprising one of the separate elements to be used in the method. If present, a second container may comprise a lysis buffer. The kit may alternatively include a computer-type chip on which the lysis of the cell will be achieved by means of an electric current.

[0052] The kit may also have containers containing nucleotides for amplification of or hybridization to the target nucleic acid sequence which may or may not be labeled, or a container

comprising a reporter, such as a biotin-binding protein, such as avidin or streptavidin, bound to a reporter molecule, such as an enzymatic, florescent, or radionuclide label. The term "detectably labeled deoxyribonucleotide" refers to a means for identifying deoxyribonucleotide. For example, the detectable label may be a radiolabeled nucleotide or a small molecule covalently bound to the nucleotide where the small molecule is recognized by a well-characterized large molecule. Examples of these small molecules are biotin, which is bound by avidin, and thyroxin, which is bound by anti-thyroxin antibody. Other methods of labeling are known to those of ordinary skill in the art, including enzymatic, fluorescent compounds, chemiluminescent compounds, phosphorescent compounds, and bioluminescent compounds.

[0053] In another embodiment the invention provides a way of screening for compounds or identifying compounds which may cause a dermatitis. In this method, cells of the skin, such as epidermal cells, including keratinocytes and melanocytes, or dermal cells, such as fibroblasts, are contacted with a test compound under conditions which would induce a dermatitis reaction. The conditions under which contact is made are variable and will depend upon the type of compound, the type and amount of cells in the skin to be tested, the concentration of the compound in the sample to be tested, as well as the time of exposure to the compound. The skill in the art in determining the proper conditions under which a compound may cause a dermatitis are known and would require only routine experimentation, if any. The skin cells may be isolated using the techniques described above, *e.g.* by scraping or tape stripping, the cells may then be exposed to the test compound *in vitro*. Alternatively, cultured skin cells or skin constructs may be used. For example, skin cells may be cultured from any source under standard cell culture conditions on a solid or semi-solid support until they become sufficiently confluent. Upon confluence or subconfluence the cells are then exposed to the test compound. Polynucleotides are then isolated from the cells which have been exposed to the compound and quantitated as described above. For example, and not by way of limitation, the cells may be isolated by the tape or scraping method above and mRNA isolated. The mRNA is then quantified using the probes for particular cytokines. Alternatively, the mRNA may be RT-PCR prior to detection of the polynucleotide. As described above, quantitation of a polynucleotide encoding a cytokine is indicative of dermatitis, for example an increase in IL-4 compared to a standard sample is indicative of ACD and an increase in IL-8 without an increase in IL-4 or IL-13 is indicative of ICD.